Sulfur-induced Polioencephalomalacia in Sheep: Some Biochemical Changes

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ABSTRACT

The effect of high dietary sulfur (S) supplementation on blood thiamine (B_1) concentration, biochemical indices of liver, muscle and kidney damage and selected plasma electrolytes was studied in six sheep. Three of these sheep received an additional 230 mg thiamine/kg diet (Group 2). After approximately 2.5-3 weeks on this diet, all three sheep in the non-B_1-supplemented group (Group 1) showed loss of appetite and developed mild neurological signs: depression, intermittent signs of excitation and head pressing. Increases in blood B_1 concentration and plasma creatine kinase (CK) and aspartate aminotransferase (AST) were observed during this time in all affected animals. Clinical signs lasted only for two to five days. Sheep in group 2 were clinically normal throughout the experiment, but all of these animals also had elevated blood B_1 concentrations and plasma CK activity at the 3 wk sampling. Plasma magnesium concentrations of group 1 sheep were elevated at the 2.5-3 wk and 6 wk samplings but they declined significantly ($p < 0.05$) to low normal levels thereafter. Magnesium concentrations of group 2 sheep were low at the beginning but progressively increased during the course of the experiment. At necropsy, brain lesions suggestive of polioencephalomalacia (PEM) were observed in all sheep but were most marked in group 1. It is speculated that PEM may be caused by a direct toxic effect of S, S metabolites or B_1 antimetabolites in the brain rather than by an *in vivo* B_1 deficiency *per se*.

RÉSUMÉ

Cette expérience portait sur deux groupes de trois agneaux et elle visait à étudier l'effet de l'ajout de beaucoup de soufre à leurs aliments, sur la teneur de leur sang en thiamine et sur les indices biochimiques reliés aux lésions hépatiques, musculaires et rénales, ainsi que sur certains électrolytes plasmatiques. Les agneaux du deuxième groupe reçurent en plus 230 mg de thiamine/kg d'aliments. Au bout d'environ trois semaines, les sujets du premier groupe affichèrent de l'anorexie et manifestèrent un peu de dépression, ainsi que des épisodes de légère excitation et de tendance à pousser au mur. Ces signes cliniques ne durèrent que de deux à cinq jours et on enregistra à ce stade, chez les trois agneaux, une élévation de la concentration du sang en thiamine et de celle du plasma en créatine-kinase et en aspartate aminotransférase. Les sujets du deuxième groupe demeurèrent normaux, tout au long de l'expérience, mais ils affichèrent aussi une élévation de la concentration sanguine en thiamine et de l'activité de la créatine-kinase plasmatique, lors de l'échantillonnage de la troisième semaine. Les sujets du premier groupe affichèrent une élévation de leur teneur plasmatique en magnésium, lors de l'échantillonnage des troisième et sixième semaines; elle diminua ensuite de façon appréciable ($p < 0,05$), pour finalement se retrouver à la limite normale inférieure. Au début de l'expérience, les agneaux du deuxième groupe affichaient une faible concentration plasmatique de magnésium, mais elle augmenta ensuite graduellement. Lors de la nécropsie, le cerveau de tous les agneaux présentait des lésions suggestives de polioencéphalomalacie, mais elles se révélèrent plus prononcées chez les sujets du premier groupe. Il semble par conséquent que la polioencéphalomalacie pourrait résulter d'un effet toxique du soufre, de ses métabolites ou des antimétabolites de la thiamine, sur le cerveau, plutôt que d'une déficience *per se* de thiamine, *in vivo*.

INTRODUCTION

Polioencephalomalacia (PEM) is a neurological disease of ruminants. It is commonly associated with feeding a high level of concentrate and/or a low amount of roughage (1). Recent studies have shown that diets (2) or drinking water (3,4) containing high levels of sulfates also predispose cattle to PEM, but little is known of the mechanisms or the biochemical changes associated with sulfur (S)-induced PEM in ruminants. Although biochemical findings on experimental and field cases of PEM are consistent with lowering of thiamine (B_1) status, there is no complete agreement in the literature that the blood B_1 levels are always reduced in affected animals. Studies on PEM have shown that blood B_1 levels may be decreased (5) while other reports have shown them to be within normal limits (6,7) or even elevated (8). The reason for such discrepancy is not clear but if PEM is induced by analogues such as amprolium or by B_1 antimetabolites blood B_1 concentrations may not always be low.

Magnesium (Mg) is a cofactor of several B_1-containing enzymes (9). Metabolic interactions between min-

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erals, and between minerals and other components in the diet and body fluids are well recognized (10). Changes in plasma concentration of the electrolytes sodium (Na), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg) and chloride (Cl) have been reported in naturally occurring PEM (11) and in amprolium-induced B_1 deficiency in sheep (6) and horses (12). Such information is lacking in S-induced PEM in ruminants. Excess S in the diet has been shown to induce muscular dystrophy in ruminants by increasing excretion of selenium (Se) in urine (13). Recent studies have shown an association between B_1 deficiency and hepatorenal syndrome in humans (14). The objective of the present study was to induce PEM experimentally in sheep by feeding high S and to examine changes in blood B_1 concentration, certain plasma electrolyte concentrations and biochemical indices of liver, muscle and kidney damage during the progression of the disease.

MATERIALS AND METHODS

ANIMALS, DIETS AND BIOCHEMICAL ANALYSES

Six, eight week old lambs of nondescript breed of mean body weight 12-15 kg were used. All lambs were fed a barley-soybean meal-alfalfa based diet containing either high (H) S (0.63%) — basal (L) B_1 (13.7 mg/kg DM) (Group 1, n = 3) or HS-HB_1 (230 mg/kg DM of B_1) (Group 2, n = 3). Measurement of blood B_1 (15) and plasma concentrations of creatine kinase (CK) (16), aspartate aminotransferase (AST) (17), γ-glutamyl transferase (GGT) (18) creatinine (CRT) (19), urea (20), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), were carried out using a Discrete Analyzer with Continuous Optical Scanning (DACOS, Coulter Electronics Inc. Hialeah, Florida) prior to initiation of the trial, at every 3 wk period thereafter, and on days when an animal showed neurological signs until the termination of the experiment. Since these animals were a part of a bile collection experiment, after 8 wks on the diets, ligation of the bile duct, and cannulation of the gall bladder and mid-third of duodenum were performed on all sheep as described by Caple and Heath (21) except sheep #81 in group 2. Clinical appearance and vital neurological signs were monitored daily. The experiments followed the guidelines of the "Guide to the Care and Use of Experimental Animals" of the Canadian Council on Animal Care.

STATISTICS

Statistical analysis was carried out using General Linear Model Analysis of Variance (GLM ANOVA) from microcomputer package [Number Cruncher Statistical System (NCSS)]. Fisher LSD (22) test was used to determine statistical significance of means between the treatment groups.

RESULTS

CLINICAL SIGNS

All animals in group 2 which received supplemental B_1 appeared clinically normal throughout the experiment. In contrast, all three sheep fed the HS-LB_1 diet (Group 1) showed loss of appetite and mild neurological signs such as mild depression, intermittent signs of excitement and head pressing within 2-3 wk. Two sheep recovered completely within two to five days but sheep #47 died suddenly on day 37 of the experiment. No signs of illness were observed in the other two animals in the group during the remainder of the experiment. Necropsy and/or histological findings indicative of PEM, such as cortical and mid-brain focal necrosis (23) were seen in all sheep, but the lesions were most severe in sheep #47 and in the remaining group 1 sheep (Olkowski *et al,* unpublished results).

TABLE I. Changes in Blood Thiamine (B_1) Concentration, and Plasma Activities of Creatine Kinase (CK), Aspartate Amino Transferase (AST) and γ-glutamyl Transferase (GGT) in Sheep Fed High Sulfur with (Group 2) or without (Group 1) Supplemental B_1 in the Diet

	Group 1[ab] High S — Basal B_1 Diet Time of Sampling (wk)					Group 2[b] High S - High B_1 Diet Time of Sampling (wk)				
	0	2.5-3	6	9	12	0	3	6	9	12
Blood B_1 concentration (μg/L)	51[d] ± 31	207[c] ± 28	73[d] ± 4	50[d] ± 1	44[d] ± 11	57[d] ± 4	742[c] ± 284	97[d] ± 4	89[d] ± 3	90[d] ± 2
Plasma CK[e] activity (U/L)	312[cd] ± 109	513[c] ± 145	386[cd] ± 104	276[cd] ± 162	118[d] ± 12	245 ± 14	750 ± 482	654 ± 333	93 ± 39	280 ± 137
Plasma AST[e] activity (U/L)	91[d] ± 1	112[d] ± 11	91[d] ± 4	300[c] ± 166	149[cd] ± 11	106 ± 21	324 ± 240	144 ± 9	73 ± 22	287 ± 126
Plasma GGT[e] activity (U/L)	73[d] ± 5	63[d] ± 6	61[d] ± 4	449[c] ± 4	470[c] ± 44	68[d] ± 2	59[d] ± 4	60[d] ± 5	159[d] ± 8	385[c] ± 43

[a] Number of animals sampled from each group was three except at 6, 9 and 12 wk in group 1 when only two animals were sampled. Sheep #47 from this group died on day 37 of the experiment

[b] Results are expressed as mean ± SD

[cd] Means within a row and within treatment followed by different letter superscripts are significantly different ($p < 0.05$)

[e] Reference values for enzymes CK, AST and GGT set by the Clinical Pathology Laboratory, Western College of Veterinary Medicine, University of Saskatchewan are < 350, 48 to 128, and < 70 U/L respectively

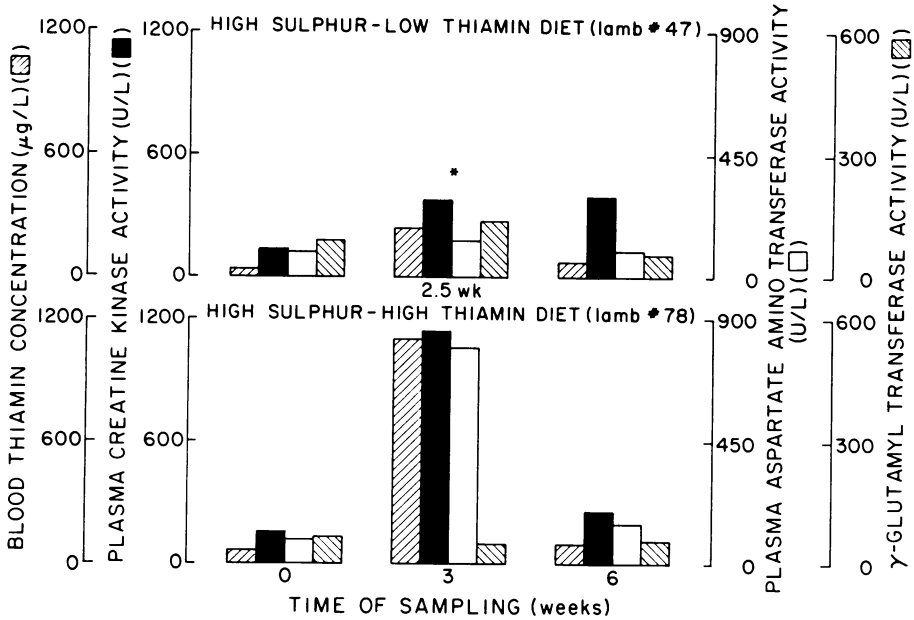

Fig. 1. Changes in blood thiamine concentrations and activities of creatine kinase, aspartate aminotransferase and γ-glutamyl transferase in plasma of a sheep given high sulfur-low thiamine diet (sheep #47) and one given high sulfur-high thiamine diet (sheep #78). The asterisk (*) indicates the time when neurological signs were observed in sheep #47.

BLOOD THIAMINE AND PLASMA ENZYME CHANGES

Blood B_1 concentrations of all lambs were within the reference limits (5) prior to the start of the experiment (Table I) but two sheep (#47 and #72) in group 1 which exhibited neurological signs had increased levels of up to 242 and 173 μg/L at the height of signs (2-3 wk). Aspartate aminotransferase activity was increased in all lambs in group 1 at this time but CK activity was increased only in two lambs. These changes were most marked in sheep #47 (Fig. 1). Samples taken at 3 wk from apparently clinically normal sheep in group 2 also showed markedly elevated blood B_1 concentration (Table I). Plasma CK was elevated in two of three lambs in this group. These changes were most marked in lamb #78 (Fig. 1). Plasma AST activity was also markedly elevated in this lamb during this time. Plasma GGT activity remained within normal limits in all six animals during the initial 6 wk period.

Blood B_1 concentration of lambs in both groups returned to within normal limits after 3 wk, but plasma CK was elevated even at 6 wk. Both CK and AST activities fluctuated during the remainder of the experiment. From 9 wk onwards GGT activities were increased in all sheep except lamb #81 (Group 2) which was not cannulated. Plasma creatinine and urea levels of all animals remained within the normal limits of 69-105 μmol/L and 0-10 mmol/L respectively.

The blood B_1 concentration of a sample from sheep #47 in group 1, taken 24-48 h prior to its death was within the normal range, but plasma CK activity was elevated (588 U/L). Both AST and GGT activities of this animal were within normal limits at this time.

PLASMA ELECTROLYTES

Changes in plasma concentrations of Na, K, Cl, Mg, Ca and P of sheep in both groups during the course of the study are given in Table II. Potassium concentrations remained within reference limits (4.6-7.0 mmol/L) in all lambs in group 1 but all lambs in group 2 exhibited hyperkalemia (up to 10 mmol/L) during week 3. This persisted in two of three animals until 6 wk but decreased thereafter. Changes in Na were minor but Cl concentrations declined significantly to below normal level at 9 wk in all group 2 sheep.

Among group 1 sheep, plasma Mg concentrations increased to above normal levels during the 2-3 and 6 wk samplings but declined significantly ($p < 0.05$) to low marginal levels thereafter. On the other hand, Mg concentrations of sheep in group 2 were low at the beginning of the experiment (normal range = 0.90-1.26 mmol/L) but increased during the course of the experiment. Slightly above normal levels were detected at the 9 wk sampling.

Plasma Ca concentrations remained within normal limits in all sheep throughout the course of the experiment. Plasma P concentrations were above normal (normal range 0.82-2.66) in all animals at the onset of the experiment. Increases in plasma P were observed in both groups during the initial 6 wk. The levels of P in group 1 sheep declined thereafter but the plasma P concentration of lambs in group 2 continued to increase throughout the experiment and high levels were observed at the termination of the study.

DISCUSSION

The present study has shown that PEM can be experimentally produced in sheep given high levels of S in the diet. This confirms observations recorded from field investigations that PEM can be caused by consumption of either high S (as sulfate) in the ration (2) or in the water (3,4,24). This is also consistent with the finding that *in vitro* sulfite is capable of cleaving B_1 in aqueous solutions (25). However Edwin *et al* (26) were unable to produce PEM in young sheep by feeding 15 g of sodium sulfite for up to one year. In the present study excess S was fed only as sulfates and it is not known whether the ionic configuration of this form of S had an influence on the development of PEM. In the present study, neurological signs indicative of B_1 deficiency were not observed in B_1-supplemented sheep. However, brain lesions suggestive of PEM (Olkowski *et al*, unpublished results), were seen in all three sheep supplemented with B_1 but the lesions were far less severe (23). This suggests that factors other than deficiency of B_1 may be responsible for the development of brain pathology of sheep fed high S diets.

Increases in blood B_1 concentrations during the appearance of early neurological signs of PEM have not been

TABLE II. Plasma Electrolyte Concentration[a] of Lambs Fed High Sulfur (S)-Normal Thiamine (B_1) (Group 1) and High S-High B_1 Diets (Group 2)

Electrolyte (mmol/L)	Group 1[a] High S — Basal B_1 Diet Time of Sampling (wk)					Group 2[a] High S - High B_1 Diet Time of Sampling (wk)				
	0	2.5-3	6	9	12	0	3	6	9	12
Sodium[d]	149 ± 2	148 ± 2	147 ± 1	148 ± 3	147 ± 1	149 ± 3	146 ± 2	143 ± 3	143 ± 4	149 ± 1
Potassium[d]	5.6[bc] ± 0.6	5.6[b] ± 0.1	6.1[b] ± 0.3	4.7[c] ± 0.1	4.6[c] ± 0.3	5.7[bc] ± 0.4	7.7[b] ± 1.3	8.1[b] ± 1.2	4.7[c] ± 0.7	5.1[bc] ± 0.5
Chloride[d]	107 ± 2	107 ± 2	107 ± 1	105 ± 1	109 ± 1	106[b] ± 1	107[b] ± 1	105[b] ± 2	89[c] ± 11	104[b] ± 1
Magnesium[d]	1.0[bc] ± 0.1	1.3[b] ± 0.1	1.3[b] ± 0.2	0.9[c] ± 0.1	0.9[c] ± 0.1	0.8[c] ± 0.1	1.0[bc] ± 0.1	1.1[bc] ± 0.1	1.3[b] ± 0.3	1.1[bc] ± 0.1
Calcium[d]	2.8 ± 0.1	2.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.1
Phosphorus[d]	2.8[c] ± 0.4	3.2[bc] ± 0.2	3.6[b] ± 0.3	2.8[c] ± 0.1	2.6[c] ± 0.2	2.9[c] ± 0.3	3.1[bc] ± 0.3	3.1[bc] ± 0.4	3.7[b] ± 0.6	3.7[b] ± 0.3

[a] Results are expressed as mean ± SD. Number of animals sampled from each group was three except at 6, 9 and 12 wk in group 1 when only two animals were sampled. Sheep #47 from this group died on day 37 of the experiment

[bc] Values with different letter superscripts within a row and within treatment differ significantly ($p < 0.05$)

[d] Reference values for electrolytes sodium, potassium, chloride, magnesium, calcium and phosphorus as recognized by the Clinical Pathology Laboratory, Western College of Veterinary Medicine, University of Saskatchewan are 143 to 151, 4.6 to 7.0, 102 to 116, 0.9 to 1.26, 2.3 to 2.86, and 0.82 to 2.66 mmol/L respectively

reported previously. However above normal blood B_1 levels have been reported in clinically normal animals within 1-2 wk of feeding amprolium, a B_1 analogue, to calves (8). This author used the thiochrome method for estimation of blood B_1 concentrations and the possibility that amprolium in blood may have interfered with the assay (7) cannot be ruled out. Slight elevations of blood B_1 accompanied by corresponding increases in urine B_1 have also been reported in sheep within 2-3 wk of feeding a bracken rhizome diet (27). In the latter study a second elevation of both blood and urine B_1 levels was observed after 4-5 wk of feeding bracken. Nervous signs were not detected on either occasion. Increased excretion of B_1 in urine has been reported in calves given a high carbohydrate diet (28), or amprolium (8). We have observed (Olkowski *et al*, unpublished observations) similar increases in urine B_1 in all sheep fed high S. As expected such increases were most marked in sheep supplemented with B_1.

The relevance of the increase in blood B_1 concentrations during early stages of the disease and its relationship to the appearance of mild neurological signs as observed in the present study is not clear. This is because extremely high blood B_1 concentrations were also observed in apparently normal B_1-supplemented sheep during the same time. But sheep fed low S diets do not show such elevations in blood B_1 concentration (Olkowski *et al*, unpublished results). The origin of the elevated B_1 in the blood of sheep fed high S is not known but it is possible that leakage may have occurred from either the muscle and/or the liver since both plasma CK and AST activities were elevated. Hepatic lesions have been inferred from clinical pathology data of amprolium-induced PEM in horses (12), but it is unlikely that such marked liver damage occurred in sheep in the present study since normal GGT levels occurred during periods of CK and AST elevations. Elevated levels of both AST and GGT were observed in subsequent weeks: 9 wk and 12 wk in group 1 sheep and 9 wk in group 2 sheep. This probably occurred from a mild cholestasis as a result of the indwelling gall bladder catheter in these animals since such an elevation was not observed in the uncannulated sheep #81 in group 2.

Although muscle does not store B_1 to any great extent, a marked elevation in plasma B_1 due to leakage from muscle is still a possibility because of the large muscle mass in the body. Increases in plasma CK have been previously reported in amprolium-induced PEM in calves (8) but only during periods of violent clonic spasms. Blood B_1 concentrations of such animals were only slightly elevated. But the possibility that blood B_1 concentrations may have peaked in these animals prior to measurement of CK cannot be discounted. Evidence of cardiac lesions, and associated functional cardiac impairment have been reported in B_1 deficiency in the cat (29) and in ruminants (30). The technique used to determine CK in the present study did not differentiate whether its origin was solely from skeletal or cardiac muscle or a combination of both. Creatine kinase is a cytosolic enzyme in muscle. High plasma CK values are indicative of either an increase in muscle cell permeability or acute damage to muscle. Increases in plasma CK have been reported in a variety of muscular disorders including Se deficiency-associated nutritional muscular dystrophy (NMD) in calves (31) and NMD in lambs (32,33). It is not known whether feeding of a high S diet to sheep precipitates an early Se deficiency but muscular dystrophy has been reported in sheep fed high S diets (13).

Increased plasma CK as observed in the present study does not exclude the possibility of nervous damage being the cause of CK release since elevations of CK have also been reported to occur in a variety of diseases of the nervous system in humans (34) and animals (35). Typical histological lesions of PEM were observed in the brains of all

sheep but the lesions were most severe in the animals not supplemented with B_1 (23). It has been postulated that in encephalomalacia, plasma CK of brain origin is not elevated since the blood brain barrier (BBB) is not altered. But starvation and stress, common occurrences during the height of nervous signs, significantly disrupt the BBB (36). Dubo *et al* (34) and Smith and Healy (35) attributed increased CK observed in nervous disorders to abnormal muscle contractions which are a part of the neurological syndrome. Although this may have occurred in group 1 lambs which exhibited mild neurological symptoms, it is unlikely to be the explanation for the rise in CK observed in group 2 lambs since neither neurological signs nor an increase in muscular activity were observed in these sheep during the time of CK rise.

Creatine kinase is eliminated rapidly from blood (37) and the half-life of CK derived from sheep is only 62 min (32). Therefore the presence of high CK levels observed in the present study at 3 and 6 wk suggests insult to muscle during these times although more frequent sampling would have confirmed whether such damage was persistent or not. However the blood B_1 concentrations of all lambs at 6 wk were normal. If blood B_1 originated from muscle, the reason for the return of blood B_1 to normal levels in spite of continued high CK levels is not known. It should be noted that most of the B_1 is associated with cellular mitochondria and only the amount of B_1 present in cytosol would leak out during permeability change or muscle damage. It can be speculated that the initial increase in muscle permeability may have resulted in leakage of almost all of the muscle cytosolic B_1 into the bloodstream leaving little or no B_1 for leakage during subsequent changes in muscle permeability. The marked increase in blood B_1 concentration observed in B_1-supplemented sheep (Group 2) probably indicates leakage from relatively higher stores of B_1 in muscle and/or liver of these sheep compared to the levels in sheep in group 1. Since both CK and blood B_1 returned to baseline values after 6 wk it is assumed that the sheep may have recovered from an initial insult caused by high S, or adapted to the high S diet by this time.

Changes in plasma electrolyte concentration observed in the present study are in agreement with previous reports on amprolium-induced PEM in cattle (8, 11). No marked changes were observed in either plasma Na or Ca concentrations. The decline in plasma Mg in group 1 sheep as opposed to an increase in group 2 sheep during the terminal stages of the experiment (week 12) is consistent with the finding that the metabolism of B_1 and Mg are interdependent (9). Magnesium is a cofactor of B_1 dependent enzymes, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and transketolase. The reason for the marked increase of plasma Mg concentration at the 2.5-3 and 6 wk samplings in group 1 sheep is not clear. Blood B_1 and plasma CK were also elevated in these animals during this time. It has been shown that animals displaying increased muscular activity may have above normal Mg concentration in blood due to a release of Mg from tissues (38). Similar increases in plasma Mg have been recorded in feedlot steers fed an all-concentrate, barley-based diet (39) and 1, 2 and 5 wk following amprolium treatment in cattle (6). The CK was not measured in the latter study. It is unlikely that Mg leaked out from the muscle since group 2 animals which showed elevated levels of CK and markedly elevated blood B_1 concentration at the 3 wk sampling showed only a marginal increase in plasma Mg.

It is notable that hyperkalemia and elevated blood B_1 occurred concurrently in group 2 sheep during week 3 and this was also observed at week 6. The highest concentration of plasma K was observed in group 1 lambs at 6 wk when plasma CK was still marginally high. Increased serum K concentrations have been reported in amprolium-treated geldings during the height of clinical signs (12). Sheep in group 1 had low-normal plasma K concentrations during the terminal stages of the present study. Low plasma K has been reported in calves during the terminal stages of PEM (40) but not in adult cattle (41). Little is known about P metabolism in animals affected with PEM. The increases in plasma P levels observed in all sheep during the initial 6 wk is probably related to rapid growth and bone turnover in young animals (42). The reason for the decline in plasma P in group 1 sheep and the increase in group 2 sheep which received B_1 supplementation is not known. Decreases in serum P levels have been previously reported in two horses receiving amprolium but these animals in addition also received Mg supplementation (12).

In conclusion, changes in several biochemical parameters observed in high S-related PEM in sheep appear to be similar to those observed in naturally occurring (11) and amprolium-induced PEM in ruminants (6,8,27) and horses (12). It is still debatable whether a localized B_1 deficient state in the brain as suggested by Loew *et al* (7) is in fact responsible for the appearance of brain lesions and signs of PEM. The present study does not support either a systemic or a localized B_1 deficiency as the cause of high S-related PEM in sheep since both blood B_1 (Table I) and brain B_1 concentrations (2.2-3.0 μg/g brain wet weight; Olkowski *et al*, unpublished results) of all sheep at the conclusion of the experiment were within the normal range [blood: 18-60 μg/L (5); brain: 1.29-1.82 μg/g wet weight (43)]. Since brain lesions suggestive of PEM were observed in both B_1-supplemented and unsupplemented groups of sheep, it seems likely that a direct toxic effect of either S, S metabolites, or B_1 antimetabolites on the brain could have been responsible for this occurrence. In view of the abundance of high sulfate water available to livestock in Saskatchewan the concept of a S-induced nervous disorder resembling a B_1 deficiency in ruminants has wide implications of economic importance. Further investigation is needed to establish the mechanisms responsible for the occurrence of this syndrome. Early identification of a subpopulation of animals at risk of development of brain damage in high S-fed animals would be extremely valuable from the standpoint of clinical management of the problem.

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